(2001. SsrA-mediated tagging in Bacillus subtilis. J. Bacteriol. 183:3885-3889) showed that the ClpXP protease is responsible for the degradation of intracellular SsrA-tagged proteins in *B. subtilis*. The instant invention provides for an enhanced protein stability via enhance protease resistance. In particular, the extracellular protease CtpA, and perhaps one or more of the major extracellular proteases of *B. subtilis,* play a role in the degradation of an extracellular, heterologous protein that was tagged by the SsrA system. It is a benefit that tagged proteins according to the present invention are more resistant to proteolysis.

Possible signal sequences that may be used

It is contemplated that any signal sequence that directs the nascent polypeptide into a secretory pathway may be used in the present invention. It is to be understood that as new signal sequences are discovered that they will be encompassed by the invention.

Signal peptides from two secretory pathways are specifically contemplated by the instant invention. The first pathway is the sec-dependent pathway. This pathway is well characterized and a number of putative signal sequences have been described. It is intended that all sec-dependent signal peptides are to be encompassed by the present invention. Specific examples include but are not limited to the AmyL and the AprE sequences. The AmyL sequence refers to the signal sequence for α-amylase and AprE refers to the AprE signal peptide sequence [AprE is subtilisin (also called alkaline protease) of B. subtilis].

The second pathway is the twin arginine translocation or Tat pathway. Similarly, it is intended that all tat-dependent signal peptides are to be encompassed by the present invention. Specific examples include but are not limited to the phoD and the lipA sequences.

Possible proteins that may be produced

The present invention is particularly useful in enhancing the production and secretion of proteins that possess non-polar or substantially non-polar

carboxy termini. Thus, it is contemplated that a protein that comprises a signal sequence and a non-polar or substantially non-polar carboxy terminus would be useful in the present invention. The protein may be homologous or heterologous. Proteins that may produced by the instant invention include, but are not limited to, hormones, enzymes, growth factors, cytokines, antibodies and the like.

Enzymes include, but are not limited to, hydrolases, such as protease, esterase, lipase, phenol oxidase, permease, amylase, pullulanase, cellulase, glucose isomerase, laccase and protein disulfide isomerase.

Hormones include, but are not limited to, follicle-stimulating hormone, luteinizing hormone, corticotropin-releasing factor, somatostatin, gonadotropin hormone, vasopressin, oxytocin, erythropoietin, insulin and the like.

Growth factors are proteins that bind to receptors on the cell surface, with the primary result of activating cellular proliferation and/or differentiation. Growth factors include, but are not limited to, platelet-derived growth factor, epidermal growth factor, nerve growth factor, fibroblast growth factors, insulin-like growth factors, transforming growth factors and the like.

Cytokines are a unique family of growth factors. Secreted primarily from leukocytes, cytokines stimulate both the humoral and cellular immune responses, as well as the activation of phagocytic cells. Cytokines include, but are not limited to, colony stimulating factors, the interleukins (IL-1 (α and β), IL-2 through IL-13) and the interferons (α, β and γ).

Human Interleukin-3 (IL-3) is a 15 kDa protein containing 133 amino acid residues. IL-3 is a species specific colony stimulating factor which stimulates colony formation of megakaryocytes, neutrophils, and macro phages from bone marrow cultures.

Antibodies include, but are not limited to, immunoglobulins from any species from which it is desirable to produce large quantities. It is especially preferred that the antibodies are human antibodies. Immunoglobulins may be from any class, i.e., G, A, M, E or D.

Possible tags that may be used

Tags may either be added to the carboxy terminus of a protein or substituted for the amino acids of the protein's carboxy terminus. If the protein has been tagged by the addition of amino acid residues the tag is preferably up to 20 additional residues preferably about 15, more preferred 1-14, even more preferred 1-11, and most preferred 1-3, wherein the last one or two amino acid residues are charged. Figure 7D depicts a protein with a tag added on to its carboxy terminus. In this depiction the tag is 14 amino acid residues long.

In the alternative, the tag may replace between 1 and 5 amino acids in the protein's carboxy terminus. In the substituted tag the amino acids are charged. In a preferred embodiment the last 5 amino acids are replaced with the tag. In another preferred embodiment the last 4 amino acids are replaced with the tag. In yet another preferred embodiment the last 3 amino acids are replaced with the tag. In a more preferred embodiment the last amino acid is replaced with the tag. In a most preferred embodiment the last 2 amino acids are replaced with the tag. Figure 7C depicts a substitution tagged protein. In this depiction the final two amino acid residues of the native protein have been replaced with two charged amino acid residues.

The charged amino acid residues may be either positively or negatively charged. The preferred negatively charged amino acids are: D or E. The preferred postively charged amino acids are: K, R or H.

The following preparations and examples are given to enable those skilled in the art to more clearly understand and practice the present invention. They should not be considered as limiting the scope and/or spirit of the invention, but merely as being illustrative and representative thereof.

In the experimental disclosure which follows, the following abbreviations apply: eq (equivalents); M (Molar); μM (micromolar); N (Normal); mol (moles); mmol (millimoles); μmol (micromoles); nmol (nanomoles); g (grams); mg (milligrams); kg (kilograms); μg (micrograms); L